Physical and Behavioral Effects of Common Endocrine Disrupting Chemicals in Western National Parks on Fish in a Synergistic Manner– Emily Guise

Introduction: Endocrine disrupting chemicals (EDCs) are chemicals that disturb endocrine processes within an organism. Mechanisms of actions and their effects vary widely (1). Due to the complexity of the endocrine feedback systems, EDCs can sometimes have the greatest influence at lower doses (1). Because EDCs are typically synthesized for practical use (such as atrazine, an herbicide), they are often made to be very stable and long lasting. Their persistent nature leads to their appearance in water runoff and precipitation, where they have been known to accumulate in ponds and sediment (2).

Certain fish species are good bioindicators for pollutant exposure (3). Because fish are a food source for many others, it is important to know the potential effects (lethal and non-lethal) of EDCs upon food webs. Fish are often exposed to a chemical milieu, thus understanding the synergistic effects of exposure is also paramount. I propose to measure the levels of polychlorinated biphenyls (PCBs), pesticides/herbicides (like atrazine and endosulfan), polycyclic aromatic hydrocarbons, and the plasticizer bisphenol A (BPA) within freshwater trophic systems and determine the transgenerational influence these EDCs have on fish physiology and behavior.

Mountainous regions are prone to EDC accumulation (4). Some high elevation areas have diurnal mountain winds, increased precipitation, and lower temperatures that increase the delivery and longevity of EDCs. Various EDCs have been measured in the Western National Parks of the US, but the synergistic effects of exposure to those pollutants have yet to be measured multi-generationally in fish. PCBs were banned in 1979 and are still found in Park samples far from their administration sites (2). National Parks were meant to be untouched nature reserves, yet are currently subjected to pollutants via uncontrollable and unavoidable sources such as precipitation. My graduate research proposes to combine the determination of the presence of pollutants with experimental studies to elucidate the impact of these chemicals on wildlife allowing me to assess the short and long-term consequences of multi-chemical exposure.

By elucidating the synergistic effects of these pollutants on some of the primary wildlife affected, we are best able to recognize and prepare for the consequences in terms of environmental changes, bioaccumulation, and policy change. If we are unable to protect our National Parks, then we will be unable to protect some of the last sources of intact nature in existence.

Methodology:

Phase 1: Seasonal samples are taken from polluted Park areas to form a profile of contaminant levels present throughout the year.

Anticipated Results: Seasonal EDC profiles will display an ecologically relevant exposure paradigm to compare to past experimental measurements done by Ackerman (2) to determine if there are any trends of the seasonal effects on contaminate levels. I might expect to see a gradual increase in the amounts of currently used EDCs (such as the plasticizer BPA) but a slight decrease in PCBs due to the 1979 ban.

Phase 2: I will begin captive animal work utilizing common fish of Park lakes (such as *Salvelinus fontinalis)*. Based on the seasonal profiles, fish will be exposed to these chemicals at ecologically relevant combinations and dosages to determine synergistic effects. I will monitor physical changes in basic and sexual morphology (such as overall size and weight, belly coloring *S. fontinalis*), hormone profiles (such as estrogen, testosterone and vitellogenin) as well as behavioral changes in memory and reproductive behaviors. The contaminant levels in the tank

will be monitored by gas chromatographic mass spectrometry, high-performance liquid chromatography, and ELISA/EIA as fit for the pollutant and sample type as described by Ackerman in (2) and Bradford in (4).

Anticipated Results: Previous studies find that individual EDCs may be found within "safe" limits (2), but I expect that the synergistic activity of multiple exposure will have unwanted effects on changes in the behavior and physical appearance of the fish. I predict increased feminization of the males, both in appearance and behavior (showing lower motivation to reproduce). I also expect fish to have higher estrogen levels in the experimental group compared to the untreated control, and no effect on testosterone. I expect the memory abilities of second generation of exposed fish will be decreased, as some EDCs can affect brain and development. Overall, I predict a decrease in the ability to reproduce and/or survive based on the treatment.

Phase 3: To determine ecological impact on exposed species, I will measure the pollutant levels within the tissues of wild-caught Park fish as well as determine their reproductive hormone profile. Comparing their physical features and behavior to the captive experiments will allow me to determine biological impacts of the pollutants on individuals within the Park.

Anticipated Results: I expect wild fish to show the same symptoms of the treated fish dependent on location and pollutant abundance. The effects on these fish may be greater/lesser depending how long they have been exposed.

Phase 4: I will mimic the current increasing contamination trends within the seasonal profiles by exposing laboratory fish to increasing levels of contamination until reaching levels predicted in 50 years (if we continue our current trend) and measure the physical effects.

Anticipated Results: I expect the fish will either die from the high levels of pollutants or become unable to survive. Either method impacts the individual and population health of National Park fish and the supported ecosystem.

Preferred Institution: My preferred institution, Oregon State University, has previously collaborated with the National Park Service (NPS) and the Dept. of Fisheries and Wildlife (DOFW) to carry out preliminary work measuring pollutant levels in lakes and sediment (2,4). Here, I will have all the equipment and expertise to measure these pollutants.

Broader Impacts: Working closely with NPS and DOFW will allow me to share my results with Park Officials and participate in fostering management strategies and educational outreach. I plan to research Crater Lake National Park and they have a Science Education Center with programs for elementary and high school students. Here, I can design educational materials for students to learn about the problems with EDCs in our Parks and our own backyards.

Due to the large amount of sampling required for this study, I will recruit undergraduate students to assist me. As a woman in science, I understand the importance of increasing the involvement of underrepresented groups. Therefore, I will recruit undergraduates these groups to assist me in order to foster their interest in science and research.

Citations:

1. Saaristo, M. et al. (2013). An androgenic agricultural contaminant impairs female reproductive behavior in a freshwater fish. PLoS ONE, 8(5), e62782.
2. Ackerman, L. K. et al. (2008). Atmospherically deposited PBDEs, pesticides, PCBs, and PAHs in western U.S. National Park fish: concentrations and consumption guidelines. Environmental Science & Technology, 42(7), 2334-2341.
3. Spearow, J. L. et al. (2010). Environmental contaminant effects of juvenile striped bass in the San Francisco estuary, California USA. Environmental Toxicology and Chemistry, 30(2), 393-402.
4. Bradford, D. F. et al. (2013). Temporal and spatial variation of atmospherically deposited organic contaminants at high elevation in Yosemite National Park, California, USA. Environmental Toxicology and Chemistry, 32(3), 517-525.

Personal Statement for Emily Guise

How I became a Scientist: I wasn't supposed to be here. I was supposed to be a doctor or an engineer. At least that's what everyone told me I *should* be. I'd always been interested in understanding how things work, and I wanted to be a capable professional. After graduating high school in 3 years, I headed to NC State to be an electrical engineer, but quickly realized that I craved more scientific theory. I realized I craved more human contact and a collaborative environment to have a fulfilling profession. Thus, I transferred to Radford University (RU) to be a nurse. Here, during an anatomy and physiology (A&P) class, my passion was lit. I was, and still am, **amazed** at how the body works. I had a nutrition professor that used to say, "Our bodies are ancient", and I am reminded of that constantly. It is not a stretch to move from being interested in engineering mechanisms to physiological mechanisms, but I am more fulfilled with the biological context and theory.

In an effort to fuel this passion, I began attending lab meetings for the RU Ecophysiology Lab. The semester after taking A&P, I switched my major to Biology. During a literature discussion, I discovered endocrine disrupting chemicals (EDCs), chemicals that alter normal endocrine function. One particular article intrigued me; it concerned an EDC called trenbolone, which is used as an anabolic steroid for growth promotion in beef cattle. Trenbolone is found in agricultural runoff and could affect freshwater fish.

My interest drove me to design my own experiment and address gaps in the current research on trenbolone. To fund my experiment I successfully applied for the Summer Undergraduate Research Fellowship through the Office of Undergraduate Research and Scholarship, the Highlander-in-Action Grant through the Scholar-Citizen Initiative, and the Biology Research Award. During the summer of 2014, with my RU funding success, I set up a captive fish lab, took water samples from ponds near cattle dosed with trenbolone, and applied those ecologically-relevant levels of trenbolone to *Gambusia holbrooki,* fish that are stocked in cow ponds as a natural mosquito control method. It's safe to say that I have found a profession I desire for a fulfilled career. I was supposed to be a doctor, but I was meant to be a scientist, thus I will be a doctor, just one with a PhD instead of an MD.

Giving Back: Studying EDCs matters as EDCs are ubiquitous. Many have heard of BPA and know it's "bad", but I've found that most people don't know why. Studying EDCs has lit an indignation within me that is not to be withheld: scientific understanding of the impacts on our health and our environment are necessary. From my passion and interests comes knowledge that I feel must be shared with those who are unknowingly impacted. My short-term goals are to inform as many as I can, especially laypeople, and parents who are trying to do what's best for their families' health. I have already begun my scientific outreach having presented at a local Women's and Minorities Health Fair to increase awareness of EDCs in our homes, and what steps can be taken to reduce exposure. I also formally presented my research at the RU Summer Undergraduate Research Forum this past fall.

My long-term goal is policy change through sound science. I want to change the way that the chemicals we use are vetted *before* they arrive on your plate and alter your body's functions. Thus far, portions of my presentations continue to address ways to influence policy change in accordance with sound science as well as increase awareness amongst policy makers. By furthering my education, I will gain credibility and continue to take part in scientific and

educational outreach by speaking at health fairs and K-12 school classrooms, while advocating for environmental health.

As a passionate researcher, I take every opportunity to share the excitement of science. As a campus tour guide for prospective students, I share my own research experiences on every tour to show others how easily undergraduate students can be involved in research regardless of major. I also take part in the Radford University Science Days throughout the semester. For Science Day, RU hosts elementary school children to show the many wonders of being a scientist. As part of Science Day, I have recruited and trained RU students to give tours of our greenhouse, with the goal of inspiring the young scientists to ask questions. We go over the various types of plants and their evolutionary purposes for the different phenotypes.

I believe that those who are able to question everything around them will go the farthest, and as an upperclassman, I strive to promote such thoughts in underclassman, especially those interested in research. Therefore, I am a TA for a university retention course for college freshman that are interested in getting involved in research. I work to help them determine their interests, how to ask questions, and guide them into research projects as early as their second semester freshman year. Initially, scientific papers can be intimidating. Personally, when I attending my first lab meeting I had my own case of imposter syndrome, and as a mentor to these up-and-coming researchers I actively share my own experiences and provide support as they begin their own research experiences.

As part of my beliefs of an informed society, I am involved in the Scholar-Citizen Initiative (SCI) here at Radford University. The goal of SCI is to take the academics we learn in the university and apply them to our passion of helping the community in diverse ways. As a scientist, SCI provides means and guidance to allow me to serve our community through presentations and opportunities to reach the public. I will graduate in the spring with Scholar-Citizen Status. In parallel with SCI, I am also working with the campus Scholarly Outreach and Research Experience (SCORE) program. The SCORE office helps provide support and technology to spread information. SCORE has provided me the ability to capture a lay audience. Our SCORE director often says, “If a scientist finds out something important but only tells other scientists, does it actually matter?” I strongly agree. As scientists, we often make the mistake of only speaking to each other. It is monumentally important that we share our findings with the public, not only for influencing policy or funding, but because scientific discovery matters. There is an immense misunderstanding of how research works, and we can only express the importance of it by sharing our findings with others. Over Fall Break, I will be going to high schools in the area and speaking with students about what being a scientist really means. I want them to know that their AP Biology course is only a foundation, a stepping-stone towards the entirety of what science really is. I want to promote STEM fields as a whole and provide insight as to what one could really do making science their profession.

Research Experience: As stated previously my research experience began at RU when I began attending the Ecophysiology Lab seminar. Other than feeling way over my head, I was also more interested in the scientific topics being discussed than in anything I had been before in my entire life. That surprised me, as I attended the lab meetings thinking I wouldn’t like it, thinking I’d just try it out, then move on to nursing school, but instead, I really really liked it. The next semester I led my first paper discussion at seminar, and I can still remember the excitement and freedom of

getting to choose **any** paper I wanted; whatever paper I thought was interesting. From that point on, the literature searches didn't stop. I can spend hours going from one paper to another to another. I want to absorb all the information I can get as I learn how to ask questions, and utilize the background information upon which to ask them.

Once I finished my review of the trenbolone literature, I designed my own experiment with the hope of filling in those informational gaps by examining multigenerational effects. I then shared my plans with the students and faculty in the Ecophysiology lab, where I was able to defend my experimental choices and receive feedback on factors to consider. From this, I gained the ability to communicate and discuss experimental design, and I have continued to maintain an active discussion throughout the progress of my experiment for insight and suggestions. The ability to find information from scientific literature, discuss the current knowledge, find the gaps in that knowledge, and discuss how to fill those gaps are all skills that I have learned from this research experience. I feel that they are invaluable skills to have in graduate school and I look forward to the transition from an undergraduate researcher to a graduate researcher.

Research has exposed me to lab techniques and equipment protocols relevant to my field of study, including ELISA/EIA, preparation of solutions, tissue analysis, field sampling techniques, and computer-assisted behavioral analysis. I have worked closely with the Institutional Animal Care and Use Committee (IACUC), personally drafting the IACUC protocol for my experiment. This year, I am serving as the student representative on the IACUC committee, which has greatly increased my knowledge of the IACUC regulatory process. I have also received training with the Occupational Safety and Health Administration (OSHA) and the Collaborative Institutional Training Initiative (CITI).

The pinnacle of my research experience has been having my abstract accepted by the Society for Integrative and Comparative Biology for a poster presentation at their 2015 National conference. This event will let me make professional connections with those doing similar research aligned with my interests. I also plan to write up my results for publication. I think both of these will be beneficial experiences that will greatly prepare me for graduate school. Next May, I will travel to the Peruvian rainforest with an RU international course to do exploratory research testing levels of pollutants (mercury from local gold mining), to determine how they are affecting the tropical ecosystem including potential effects wildlife. This trip will foster international scholarly and cultural connections at the research station there.

As a graduate student, I will continue to spread my passion for science. Influenced from my own undergraduate research experience, I will provide the same research opportunities to underrepresented students at my graduate institution. I will take part in science education programs at nearby National Parks through the creation of instructional materials to inform our young scientists of the problems associated with environmental pollutants and what can be done.

Role of GRFP and Career Aspirations: I feel that my research project looking into effects of EDC pollution could have strong implications for the future of the environment and environmental policy. A Graduate Research Fellowship would be the first stepping-stone that would allow me to reach my goal of creating a healthier environment. I want to work for the EPA continuing this type of research and to be in a position to speak for sound scientific regulatory policy and public education. The GRFP will provide the support for me to achieve these professional and personal goals.